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Revised April 2008

Introduction

The E.Z.N.A.™ SQ Blood DNA Kit is designed for isolating high molecular weight genomic DNA from fresh, frozen, and anti-coagulated whole blood. The method can also be used for preparation of genomic DNA from buffy coat, bone marrow or cultured cells. The kit allows single or multiple, simultaneous processing of samples in under 90 minutes. There is no need for phenol/chloroform extractions, and time-consuming steps such as CsCl gradient ultracentrifugation are eliminated.

DNA purified using the E.Z.N.A.™ SQ Blood DNA method is ready for applications such as PCR*, Southern blotting, and restriction digestion.

Principle

E.Z.N.A.® SQ Blood DNA Kit uses a highly efficient solution based system to provide a convenient, fast, reliable and non-toxic method to isolate high molecular weight genomic DNA from whole blood or buffy coat. Red blood cells are first lysed with ERL buffer, followed by lysis of the white blood cells and their nuclei in the WTL Buffer. Cellular proteins are removed by precipitation and high molecular weight genomic DNA will remain in solution. High quality genomic DNA is then purified by isopropanol precipitation.

Storage and Stability

All components of the E.Z.N.A.® SQ Blood DNA Kit should be stored at 22°C-25°C. Under cool ambient conditions, a precipitate may form in the Buffer WTL. In case of such an event, heat the bottle at 55°C to dissolve.

Expiration Date: All E.Z.N.A.® SQ Blood DNA Kit components are guaranteed for at least 24 months from the date of purchase when stored at 22-25°C

* The PCR process is covered by U.S. Patents 4,683,195 and 4,683,202 (and international equivalents) owned by Hoffmann-LaRoche, Inc.

Kit Contents

Product	D5032-00	D5032-01	D5032-02	D5032-03
Total Blood Volume	10 mL	50 mL	150 mL	300 mL
ERL Buffer (10 x)	5 mL	25 mL	80 mL	2 x 80 mL
Buffer WTL	10 mL	50 mL	150 mL	300 mL
PCP Buffer	4 mL	20 mL	60 mL	120 mL
EB Buffer	5 mL	20 mL	60 mL	125 mL
RNase A	50 µL	250 µL	750 µL	1.5 mL
User Manual	1	1	1	1

Before Starting

IMPORTANT	**ERL Buffer Concentrate must be diluted with ddH2O as follows before use** **D5032-00 Add 45 mL water/bottle** **D5032-01 Add 225 mL water/bottle** **D5032-02 Add 720 mL water/bottle** **D5032-03 Add 720 mL water/bottle**

NOTE: The procedures below have been optimized for use with FRESH or FROZEN blood samples. Anti-coagulated blood, or Buffy Coat can also be used. However, DNA yield will be reduced with time of storage. In addition, leukocytes or cultured cells may be used with this procedure.

DNA Yields From Various Starting Materials

Species and Material	Amount of Starting material	Typical Yield
Human Whole Blood (Yield varies depending on the quantity of white blood cells present)	50 µL	0.3-0.6 µg
	100 µL	1-5 µg
	200 µL	3-10 µg
	300 µL	5-15 µg
	500 µL	7-23 µg
	600 µL	10-30 µg
	800 µL	12-35 µg
	1 mL	15-48 µg
	2 mL	30-90 µg
	3 mL	50-150 µg
	4 mL	65-200 µg
	5 mL	100-300 µg
	10 mL	150-600 µg
	12 mL	200-700 µg
Buffy Coat	from 300 µL blood	5-15 µg
	from 2 mL blood	25-75 µg
Mouse Whole Blood	50 µL	0.2-0.6 µg
	100 µL	0.5-1.0 µg
	200 µL	2 -5 µg
	300 µL	4–7 µg
Cultured Cells	2 x 10^6 cells	10-15 µg

Guideline for Sample and Volume of Reagents

The SQ Blood DNA system is a solution based system and the protocol can be easily modified based on the sample volume. User can change the protocol based on the following guideline:

Sample volume	Reagent Name	Reagent volume
1 x	ERL Buffer	3 x
1 x	WTL Buffer	1 x
1 x	PCP Buffer	0.33 x
1x	Isopropanol	1 x
1 x	70% ethanol	1 x
1 x	EB Buffer	0.35 x *

* For ≤300µl blood volume. Volume of EB Buffer used can be adjusted depends on the desired final concentration.

A. DNA Purification Protocol for 100µL whole blood

Materials to be supplied by user

- Microcentrifuge capable of 14,000 x g
- Nuclease-free 1.5 mL microcentrifuge tubes
- Water Bath preset at 37°C and 65°C
- Isopropanol
- 70% ethanol

1. Add 100µL whole blood (or bone marrow) to a nuclease-free 1.5 mL microcentrifuge tube containing 300µL ERL Buffer. Mix by inverting the tube a few times. Incubate 5 minutes at room temperature. Invert the tube several times during the incubation.

   NOTE: ERL Buffer is supplied as a 10x concentrate and must be diluted with ddH_2O according to bottle label or Page 4 above before use.

2. Centrifuge at 14,000 x g for 30 seconds at room temperature. Remove and discard as much supernatant as possible without disturbing the visible white pellet. Leave about 10µL of residue liquid in the tube. If the blood sample has been frozen, repeat Steps 1-2 until the pellet is white.

   Note: If some red blood cells or cell debris are still visible along with the white blood cell pellet, resuspend the white blood cell pellet and mix with 2 volumes ERL Buffer. Incubate 2 min at room temperature. Then pellet the white blood cells by repeating Step 2.

3. Vortex the tube vigorously until the white blood cells are completely re-suspended.

4. Add 100µL WTL Buffer to the tube containing the re-suspended cells. Pipet up and down to lyse the cells. The solution should become very viscous. If cell clumps are visible after mixing, incubate the solution at 37°C until the clumps cannot be seen.

5. **(Optional)** Add 0.5µL RNase A solution to the cell lysate. Mix the sample by inverting the tube 20-25 times. Incubate the mixture at 37°C for 5-10 minutes.

6. Cool the sample to room temperature.

7. Add 33µL PCP Buffer to the cell lysate.

8. Vortex vigorously at high speed for 30 seconds to mix. Some protein clumps may be visible after vortexing. Incubate in ice for 5 minutes.

9. Centrifuge at max speed for 3 minutes at room temperature. The precipitated protein will form a tight, dark brown pellet. If the pellet is not tight or visible, incubate the tube in ice for 5 minutes and repeat Step 9.

10. Transfer the supernatant to a new nuclease-free 1.5 mL centrifuge tube containing 100 µL of 100% isopropanol.

11. Gently mix the solution by inverting the tube 30-40 times.

12. Centrifuge at 14,000 x g for 1 minute at room temperature. DNA will be visible as a small white pellet.

13. Pour out the supernatant and drain the tube briefly on a clean absorbent paper towel. Add 100µL of 70% ethanol and invert the tube few times to wash the DNA pellet.

14. Centrifuge at 14,000 x g for 2 minutes at room temperature. Carefully pour off the ethanol. **Pellet may be very loose at this point, so pour slowly and watch the pellet.**

15. Invert the tube on a clean absorbent paper towel and air dry the pellet for 10-15 minutes.

16. Add 35 µL of DNA rehydration solution (Buffer EB) and vortex for 1 minute to mix.

17. Incubate sample at 65°C for 10 min. Some samples may need to incubate at 65°C for 1 hour to rehydrate DNA.

18. Store DNA at 2-8°C. For long-term storage, store at -20°C.

B. DNA Purification Protocol for 200µL whole blood

Materials to be supplied by user

- Microcentrifuge capable of 14,000 x g
- Nuclease-free 1.5 mL microcentrifuge tubes
- Water Bath preset at 37°C and 65°C
- Isopropanol
- 70% ethanol

1. Add 200µL whole blood (or bone marrow) to a nuclease-free 1.5 mL microcentrifuge tube containing 600µL ERL Buffer. Mix by inverting the tube a few times. Incubate 5 minutes at room temperature. Invert the tube several times during incubation.

   NOTE: ERL Buffer is supplied as a 10x concentrate and must be diluted with ddH_2O according to bottle label or Page 4 above before use.

2. Centrifuge at 14,000 x g for 30 seconds at room temperature. Remove and discard as much supernatant as possible without disturbing the visible white pellet. Leave about 15µL of residue liquid in the tube. If the blood sample has been frozen, repeat Steps 1-2 until the pellet is white.

   Note: If some red blood cells or cell debris are still visible along with the white blood cell pellet, resuspend the white blood cell pellet and mix with 2 volumes ERL Buffer. Incubate 2 min at room temperature. Then pellet the white blood cells by repeating Step 2.

3. Vortex the tube vigorously until the white blood cells are completely re-suspended.

4. Add 200µL WTL Buffer to the tube containing the re-suspended cells. Pipet up and down to lyse the cells. The solution should become very viscous. If cell clumps are visible after mixing, incubate the solution at 37°C until the clumps cannot be seen.

5. **(Optional)** Add 1 µL RNase A solution to the cell lysate. Mix the sample by inverting the tube 20-25 times. Incubate the mixture at 37°C for 5-10 minutes.

6. Cool the sample to room temperature.

7. Add 67µL PCP Buffer to the cell lysate.

8. Vortex vigorously at high speed for 30 seconds to mix. Some protein clumps may be visible after vortexing. Incubate in ice for 5 minutes.

9. Centrifuge at max speed for 3 minutes at room temperature. The precipitated protein will form a tight, dark brown pellet. If the pellet is not tight or visible, incubate the tube in ice for 5 minutes and repeat Step 9.

10. Transfer the supernatant to a new nuclease-free 1.5 mL centrifuge tube containing 200µL of 100% isopropanol.

11. Gently mix the solution by inverting the tube 30-40 times.

12. Centrifuge at 14,000 x g for 1 minute at room temperature. DNA will be visible as a small white pellet.

13. Pour out the supernatant and drain the tube briefly on a clean absorbent paper towel. Add 200µL of 70% ethanol and invert the tube a few times to wash the DNA pellet.

14. Centrifuge at 14,000 x g for 2 minutes at room temperature. Carefully pour off the ethanol. **Pellet may be very loose at this point, so pour slowly and watch the pellet.**

15. Invert the tube on a clean adsorbent paper towel and air dry the pellet for 10-15 minutes.

16. Add 65 µL of DNA rehydration solution (Buffer EB) and vortex for 1 minute to mix.

17. Incubate sample at 65°C for 10 min. Some sample may need to incubate at 65°C for 1 hour to rehydrate DNA..

18. Store DNA at 2-8°C. For long-term storage, store at -20°C.

C. DNA Purification Protocol for 300µL whole blood

Materials to be supplied by user

- Microcentrifuge capable of 14,000 x g
- Nuclease-free 1.5 mL microcentrifuge tubes
- Water Bath preset at 37°C and 65°C
- Isopropanol
- 70% ethanol

1. Add 300µL whole blood (or bone marrow) to a nuclease-free 1.5 mL microcentrifuge tube containing 900µL ERL Buffer. Mix by inverting the tube a few times. Incubate 5 minutes at room temperature. Invert the tube several times during incubation.

   NOTE: ERL Buffer is supplied as a 10x concentrate and must be diluted with ddH_2O according to bottle label or Page 4 above before use.

2. Centrifuge at 14,000 x g for 30 seconds at room temperature. Remove and discard as much supernatant as possible without disturbing the visible white pellet. Leave about 15µL of residue liquid in the tube. If the blood sample has been frozen, repeat Steps 1-2 until the pellet is white.

   Note: If some red blood cells or cell debris are still visible along with the white blood cell pellet, resuspend the white blood cell pellet and mix with 2 volumes ERL Buffer. Incubate 2 min at room temperature. Then pellet the white blood cells by repeating Step 2.

3. Vortex the tube vigorously until the white blood cells are completely re-suspended.

4. Add 300µL WTL Buffer to the tube containing the re-suspended cells. Pipet up and down to lyse the cells. The solution should become very viscous. If the cell clumps are visible after mixing, incubate the solution at 37°C until the clumps cannot be seen.

5. **(Optional)** Add 1.5 µL RNase A solution to the cell lysate. Mix the sample by inverting the tube 20-25 times. Incubate the mixture at 37°C for 5-10 minutes.

6. Cool the sample to room temperature.

7. Add 100µL PCP Buffer to the cell lysate.

8. Vortex vigorously at high speed for 30 seconds to mix. Some protein clumps may be visible after vortexing. Incubate in ice for 5 minutes.

9. Centrifuge at max speed for 3 minutes at room temperature. The precipitated protein will form a tight, dark brown pellet. If the pellet is not tight or visible, incubate the tube in ice for 5 minutes and repeat Step 9.

10. Transfer the supernatant to a new nuclease-free 1.5 mL centrifuge tube containing 300µL of 100% isopropanol.

11. Gently mix the solution by inverting the tube 30-40 times.

12. Centrifuge at 14,000 x g for 1 minute at room temperature. DNA will be visible as a small white pellet.

13. Pour out the supernatant and drain the tube briefly on a clean absorbent paper towel. Add 300µL of 70% ethanol and invert the tube few times to wash the DNA pellet.

14. Centrifuge at 14,000 x g for 2 minutes at room temperature. Carefully pour off the ethanol. **Pellet may be very loose at this point, so pour slowly and watch the pellet.**

15. Invert the tube on a clean adsorbent paper towel and air dry the pellet for 10-15 minutes.

16. Add 100 µL of DNA rehydration solution (Buffer EB) and vortex for 1 minute to mix.

17. Incubate sample at 65°C for 10 min. Some samples may need to incubate at 65°C for 1-2 hour to rehydrate DNA..

18. Store DNA at 2-8°C. For long-term storage, store at -20°C.

D. DNA Purification Protocol for 600µL whole blood

Materials to be supplied by user

- Microcentrifuge capable of 14,000 x g
- Nuclease-free 2.0 mL microcentrifuge tubes
- Water Bath preset at 37°C and 65°C
- Isopropanol
- 70% ethanol

1. Add 600µL whole blood (or bone marrow) to a nuclease-free 2.0 mL microcentrifuge tube containing 1.2mL ERL Buffer. Mix by inverting the tube a few times. Incubate 5 minutes at room temperature. Invert the tube several times during incubation.

   NOTE: ERL Buffer is supplied as a 10x concentrate and must be diluted with ddH_2O according to bottle label or Page 4 above before use.

2. Centrifuge at 14,000 x g for 30 seconds at room temperature. Remove and discard supernatant containing lysed red blood cells. Leave behind any supernatant that appears to contain non-lysed red blood cells..

3. Vortex the tube vigorously until the cell pellet is completely resuspended.

4. Add 1.2 mL ERL buffer to the tube and mix well by inverting the tube a few times. Incubate at room temperature for 2 minutes.

5. Centrifuge at 14000 x g for 30 seconds at room temperature. Remove and discard as much supernatant as possible without disturbing the visible white pellet. Leave about 15-20µL of residue liquid in the tube. If the blood sample has been frozen, repeat Steps 1-5 until the pellet is white.

   Note: If some red blood cells or cell debris are still visible along with the white blood cell pellet, resuspend the white blood cell pellet and mix with 2 volumes ERL Buffer. Incubate 2 min at room temperature. Then pellet the white blood cells by repeating Step 2.

6. Add 600µL WTL Buffer to the tube containing the re-suspended cells. Pipet up and down to lyse the cells. The solution should become very viscous. If cell clumps are visible after mixing, incubate the solution at 37°C until the clumps cannot be seen.

7. **(Optional)** Add 3 µL RNase A solution to the cell lysate. Mix the sample by inverting the tube 20-25 times. Incubate the mixture at 37°C for 5-10 minutes.

8. Cool the sample to room temperature.

9. Add 200µL PCP Buffer to the cell lysate.

10. Vortex vigorously at high speed for 30 seconds to mix. Some protein clumps may be visible after vortexing. Incubate in ice for 5 minutes.

11. Centrifuge at max speed for 3 minutes at room temperature. The precipitated protein will form a tight, dark brown pellet. If the pellet is not tight or visible, incubate the tube in ice for 5 minutes and repeat Step 11.

12. Transfer the supernatant to a new nuclease-free 2.0 mL centrifuge tube containing 600µL of 100% isopropanol.

13. Gently mix the solution by inverting the tube 30-40 times.

14. Centrifuge at 14,000 x g for 1 minute at room temperature. DNA will be visible as a small white pellet.

15. Pour of the supernatant and drain the tube briefly on a clean absorbent paper towel. Add 600µL of 70% ethanol and invert the tube a few times to wash the DNA pellet.

16. Centrifuge at 14,000 x g for 2 minutes at room temperature. Carefully pour off the ethanol. **Pellet may be very loose at this point, so pour slowly and watch the pellet.**

17. Invert the tube on a clean adsorbent paper towel and air dry the pellet for 10-15 minutes.

18. Add 100 µL of DNA rehydration solution (Buffer EB) and vortex for 1 minute to mix.

19. Incubate sample at 65°C for 10 min. Some samples may need to incubate at 65°C for 1-2 hour to rehydrate DNA..

20. Store DNA at 2-8°C. For long-term storage, store at -20°C.

E. DNA Purification Protocol for 800 µL whole blood

Materials to be supplied by user

- Centrifuge capable of 2,000 x g
- Nuclease-free 15 mL centrifuge tubes
- Water baths preset at 37°C and 65°C
- Paper towels
- Isopropanol (100%)
- 70% ethanol

19. Add 800 µL whole blood (or bone marrow) to a nuclease-free 15 mL centrifuge tube containing 2.4 mL ERL Buffer. Mix by inverting the tube a few times. Incubate 5 minutes at room temperature. Invert the tube several times during the incubation.

    NOTE: ERL Buffer is supplied as a 10x concentrate and must be diluted with ddH_2O according to bottle label or Page 4 above before use.

20. Centrifuge at 2,000 x g for 5 minutes at room temperature. Remove and discard as much supernatant as possible without disturbing the visible white pellet. Leave about 50 µL of residue liquid in the tube. If the blood sample has been frozen, repeat Steps 1-2 until the pellet is white.

    Note: If some red blood cells or cell debris are still visible along with the white blood cell pellet, resuspend the white blood cell pellet and mix with 2 volumes ERL Buffer. Incubate 2 min at room temperature. Then pellet the white blood cells by repeating Step 2.

21. Vortex the tube vigorously until the white blood cells are completely resuspended.

22. Add 800µL WTL Buffer to the tube containing the resuspended cells. Pipet up and down to lyse the cells. The solution should become very viscous. If cell clumps are visible after mixing, incubate the solution at 37°C until the clumps cannot be seen.

23. **(Optional)** Add 4 µL RNase A solution to the cell lysate. Mix the sample by inverting the tube 20-25 times. Incubate the mixture at 37°C for approximately 10 minutes.

24. Cool the sample to room temperature.

25. Add 270 µL PCP Buffer to the cell lysate.

26. Vortex vigorously at high speed for 30 seconds to mix. Some protein clumps may be visible after vortexing.

27. Centrifuge at 2,000 x g or higher for 5 minutes at room temperature. The precipitated protein will form a tight, dark brown pellet. If the pellet is not tight or visible, repeat Step 8, incubate the tube in ice for 5 minutes, and then repeat Step 9.

28. Transfer the supernatant to a new nuclease-free 15 mL centrifuge tube containing 800 µL of 100% isopropanol. Do not transfer the protein pellet.

29. Gently mix the solution by inverting the tube 40-50 times.

30. Centrifuge at 2,000 x g for 3 minutes at room temperature. DNA will be visible as a small white pellet.

31. Pour out the supernatant and drain the tube briefly on an absorbent paper towel. Add 800 µL of 70% ethanol and invert the tube a few times to wash the DNA pellet.

32. Centrifuge at 2,000 x g for 2 minutes at room temperature. Carefully pour out the ethanol. **Pellet may be very loose at this point, so pour slowly and be careful not to pour out the pellet.**

33. Invert the tube on an absorbent paper towel and air dry the pellet for 10-15 minutes.

34. Add 100 µL of Buffer EB and vortex for 1 minute to mix.

35. Incubate sample at 65°C for 1 hour to rehydrate DNA. Gently shake tube several times during incubation to disperse the DNA. Some samples may need to incubate at room temperature overnight to rehydrate DNA.

36. Store DNA at 2-8°C. For long-term storage, store at -20°C.

F. DNA Purification Protocol for 1 mL Whole Blood

Materials to be supplied by user

- Centrifuge capable of 14,000 x g
- Nuclease-free 1.5 mL microcentrifuge tubes
- Water baths preset at 37°C and 65°C
- Paper towels
- Isopropanol (100%)
- 70% ethanol

1. Add 1 mL whole blood (or bone marrow) to a nuclease-free 15 mL centrifuge tube containing 3 mL ERL Buffer. Mix by inverting the tube a few times. Incubate 5 minutes at room temperature. Invert the tube several times during incubation.

   NOTE: ERL Buffer is supplied as a 10x concentrate and must be diluted with ddH_2O according to bottle label or Page 4 above before use.

2. Centrifuge at 2,000 x g for 5 minutes at room temperature. Remove and discard as much supernatant as possible without disturbing the visible white pellet. Leave about 100 µL of residue liquid in the tube. If the blood sample has been frozen, repeat Steps 1-2 until the pellet is white.
   Note: If some red blood cells or cell debris are still visible along with the white blood cell pellet, resuspend the white blood cell pellet and mix with 2 volumes ERL Buffer. Incubate 2 min at room temperature. Then pellet the white blood cells by repeating Step 2.

3. Vortex the tube vigorously until the white blood cells are completely resuspended.

4. Add 1 mL WTL Buffer to the tube containing the resuspended cells. Pipet up and down to lyse the cells. The solution should become very viscous. If cell clumps are visible after mixing, incubate the solution at 37°C until the clumps cannot be seen.

5. **(Optional)** Add 5 µL RNase A solution to the cell lysate. Mix the sample by inverting the tube 20-25 times. Incubate the mixture at 37°C for approximately 10 minutes.

6. Cool the sample to room temperature.

7. Add 335 µL PCP Buffer to the cell lysate.

8. Vortex vigorously at high speed for 30 seconds to mix. Some protein clumps may be visible after vortexing.

9. Centrifuge at 2,000 x g or higher for 5 minutes at room temperature. The precipitated protein will form a tight, dark brown pellet. If the pellet is not tight or visible, repeat Step 8, incubate the tube in ice for 5 minutes, and then repeat Step 9.

10. Transfer the supernatant to a new nuclease-free 15 mL centrifuge tube containing 1 mL of 100% isopropanol. Do not transfer the protein pellet.

11. Gently mix the solution by inverting the tube 40-50 times.

12. Centrifuge at 2,000 x g for 3 minutes at room temperature. DNA will be visible as a small white pellet.

13. Pour out the supernatant and drain the tube briefly on an absorbent paper towel. Add 1 mL of 70% ethanol and invert the tube a few times to wash the DNA pellet.

14. Centrifuge at 2,000 x g for 2 minutes at room temperature. Carefully pour out the ethanol. **Pellet may be very loose at this point, so pour slowly and be careful not to pour out the pellet.**

15. Invert the tube on a clean adsorbent paper towel and air dry the pellet for 10-15 minutes.

16. Add 100 µL of Buffer EB and vortex for 1 minute to mix.

17. Incubate sample at 65°C for -2 hour to rehydrate DNA. Gently shake tube several times during incubation to disperse DNA. Some samples may need to incubate at room temperature overnight to rehydrate DNA.

18. Store DNA at 2-8°C. For long-term storage, store at -20°C.

G. DNA Purification Protocol for 2 mL Whole Blood

Materials to be supplied by user

- Centrifuge capable of 2,000 x g
- Nuclease-free 15 mL centrifuge tubes
- Water baths preset at 37°C and 65°C
- Paper towels
- Isopropanol (100%)
- 70% ethanol

1. Add 2 mL whole blood (or bone marrow) to a nuclease-free 15 mL microcentrifuge tube containing 6 mL ERL Buffer. Mix by inverting the tube a few times. Incubate 5 minutes at room temperature. Invert the tube several times during incubation.

   NOTE: ERL Buffer is supplied as a 10x concentrate and must be diluted with ddH_2O according to bottle label or Page 4 above before use.

2. Centrifuge at 2,000 x g for 5 minutes at room temperature. Remove and discard as much supernatant as possible without disturbing the visible white pellet. Leave about 150 µL of residue liquid in the tube. If the blood sample has been frozen, repeat Steps 1-2 until the pellet is white.
   Note: If some red blood cells or cell debris are still visible along with the white blood cell pellet, resuspend the white blood cell pellet and mix with 2 volumes ERL Buffer. Incubate 2 min at room temperature. Then pellet the white blood cells by repeating Step 2.

3. Vortex the tube vigorously until the white blood cells are completely resuspended.

4. Add 2 mL WTL Buffer to the tube containing the resuspended cells. Pipet up and down to lyse the cells. The solution should become very viscous. If cell clumps are visible after mixing, incubate the solution at 37°C until the clumps cannot be seen.

5. **(Optional)** Add 10 µL RNase A solution to the cell lysate. Mix the sample by inverting the tube 20-25 times. Incubate the mixture at 37°C for approximately 10 minutes.

6. Cool the sample to room temperature.

7. Add 670 µL PCP Buffer to the cell lysate.

8. Vortex vigorously at high speed for 30 seconds to mix. Some protein clumps may be visible after vortexing.
9. Centrifuge at 2,000 x g or higher for 5 minutes at room temperature. The precipitated protein will form a tight, dark brown pellet. If the pellet is not tight or visible, repeat Step 8, incubate the tube in ice for 5 minutes, and then repeat Step 9.

10. Transfer the supernatant to a new nuclease-free 15 mL centrifuge tube containing 2 mL of 100% isopropanol. Do not transfer the protein pellet.

11. Gently mix the solution by inverting the tube 40-50 times.

12. Centrifuge at 2,000 x g for 3 minutes at room temperature. DNA will be visible as a small white pellet.

13. Pour out the supernatant and drain the tube briefly on an absorbent paper towel. Add 2 mL of 70% ethanol and invert the tube few times to wash the DNA pellet.

14. Centrifuge at 2,000 x g for 2 minutes at room temperature. Carefully pour out the ethanol. **Pellet may be very loose at this point, so pour slowly and be careful not to pour out the pellet.**

15. Invert the tube on an adsorbent paper towel and air dry the pellet for 10-15 minutes.

16. Add 200 µL of Buffer EB and vortex for 1 minute to mix.

17. Incubate sample at 65°C for 1 hour and room temperature for overnight to rehydrate DNA. Gently shake tube several times during incubation to disperse DNA.

18. Store DNA at 2-8°C. For long-term storage, store at -20°C.

H. DNA Purification Protocol for 3 mL Whole Blood

Materials to be supplied by user

- Microcentrifuge capable of 14,000 x g
- Nuclease-free 15 mL centrifuge tubes
- Water baths preset at 37°C and 65°C
- Paper towels
- Isopropanol
- 70% ethanol

1. Add 3 mL whole blood (or bone marrow) to a nuclease-free 15 mL centrifuge tube containing 9 mL ERL Buffer. Mix by inverting the tube a few times. Incubate 5 minutes at room temperature. Invert the tube several times during incubation.

   NOTE: ERL Buffer is supplied as a 10x concentrate and must be diluted with ddH_2O according to bottle label or Page 4 above before use.

2. Centrifuge at 2,000 x g for 5 minutes at room temperature. Remove and discard as much supernatant as possible without disturbing the visible white pellet. Leave about 150-200 µL of residue liquid in the tube. If the blood sample has been frozen, repeat Steps 1-2 until the pellet is white.

   NOTE: ERL Buffer is supplied as a 10x concentrate and must be diluted with ddH_2O according to bottle label or Page 4 above before use.

3. Vortex the tube vigorously until the cell pellet is completely resuspended.

4. Add 3 mL WTL Buffer to the tube containing the resuspended cells. Pipet up and down to lyse the cells. The solution should become very viscous. If cell clumps are visible after mixing, incubate the solution at 37°C until the clumps cannot be seen.

5. **(Optional)** Add 15 µL RNase A solution to the cell lysate. Mix the sample by inverting the tube 20-25 times. Incubate the mixture at 37°C for approximately 10 minutes.

6. Cool the sample to room temperature.

7. Add 1 mL PCP Buffer to the cell lysate.

8. Vortex vigorously at high speed for 30 seconds to mix. Some protein clumps may be visible after vortexing.

9. Centrifuge at 2,000 x g for 5 minutes at room temperature. The precipitated protein will form a tight, dark brown pellet. If the pellet is not tight or visible, repeat Step 8, incubate the tube in ice for 5 minutes, and then repeat Step 9

10. Transfer the supernatant to a new nuclease-free 15 mL centrifuge tube containing 3 mL of 100% isopropanol. Do not transfer the protein pellet.

11. Gently mix the solution by inverting the tube 40-50 times.

12. Centrifuge at 2,000 x g for 3 minutes at room temperature. DNA will be visible as a small white pellet.

13. Pour out the supernatant and drain the tube briefly on an absorbent paper towel. Add 3 mL of 70% ethanol and invert the tube a few times to wash the DNA pellet.

14. Centrifuge at 2,000 x g for 2 minutes at room temperature. Carefully pour out the ethanol. **Pellet may be very loose at this point, so pour slowly and be careful not to pour out the pellet.**

15. Invert the tube on an adsorbent paper towel and air dry the pellet for 10-15 minutes.

16. Add 250 µL of Buffer EB and vortex for 1 minute to mix.

17. Incubate sample at 65°C for 1 hour and room temperature for overnight to rehydrate DNA. Gently shake tube several times during incubation to disperse DNA.

18. Store DNA at 2-8°C. For long-term storage, store at -20°C.

I. DNA Purification Protocol for 4 mL Whole Blood

Materials to be supplied by user

- Centrifuge capable of 2,000 x g
- Nuclease-free 15 mL centrifuge tubes
- Water baths preset at 37°C and 65°C
- Paper towels
- Isopropanol (100%)
- 70% ethanol

1. Add 4 mL whole blood (or bone marrow) to a nuclease-free 15 mL microcentrifuge tube containing 12 mL ERL Buffer. Mix by inverting the tube a few times. Incubate 5 minutes at room temperature. Invert the tube several times during incubation.

   NOTE: ERL Buffer is supplied as a 10x concentrate and must be diluted with ddH_2O according to bottle label or Page 4 above before use.

2. Centrifuge at 2,000 x g for 5 minutes at room temperature. Remove and discard as much supernatant as possible without disturbing the visible white pellet. Leave about 300 µL of residue liquid in the tube. If the blood sample has been frozen, repeat Steps 1-2 until the pellet is white.
   Note: If some red blood cells or cell debris are still visible along with the white blood cell pellet, resuspend the white blood cell pellet and mix with 2 volumes ERL Buffer. Incubate 2 min at room temperature. Then pellet the white blood cells by repeating Step 2.

3. Vortex the tube vigorously until the white blood cells are completely re-suspended.

4. Add 4 mL WTL Buffer to the tube containing the re-suspended cells. Pipet up and down to lyse the cells. The solution should become very viscous. If cell clumps are visible after mixing, incubate the solution at 37°C until the clumps cannot be seen.

5. **(Optional)** Add 20 µL RNase A solution to the cell lysate. Mix the sample by inverting the tube 20-25 times. Incubate the mixture at 37°C for approximately 10 minutes.

6. Cool the sample to room temperature.

7. Add 1.35 mL PCP Buffer to the cell lysate.

8. Vortex vigorously at high speed for 30 seconds to mix. Some protein clumps may be visible after vortexing.

9. Centrifuge at 2,000 x g or higher for 5 minutes at room temperature. The precipitated protein will form a tight, dark brown pellet. If the pellet is not tight or visible, repeat Step 8, incubate the tube in ice for 5 minutes, and then repeat Step 9.

10. Transfer the supernatant to a new nuclease-free 15 mL centrifuge tube containing 4 mL of 100% isopropanol. Do not transfer the protein pellet.

11. Gently mix the solution by inverting the tube 40-50 times.

12. Centrifuge at 2,000 x g for 3 minutes at room temperature. DNA will be visible as a small white pellet.

13. Pour out the supernatant and drain the tube briefly on an absorbent paper towel. Add 4 mL of 70% ethanol and invert the tube few times to wash the DNA pellet.

14. Centrifuge at 2,000 x g for 2 minutes at room temperature. Carefully pour out the ethanol. **Pellet may be very loose at this point, so pour slowly and be careful not to pour out the pellet.**
15. Invert the tube on an adsorbent paper towel and air dry the pellet for 10-15 minutes.

16. Add 400 µL of Buffer EB and vortex for 1 minute to mix.

17. Incubate sample at 65°C for 1 hour and overnight at room temperature to rehydrate DNA. Gently shake tube several times during incubation to disperse DNA.

18. Store DNA at 2-8°C. For long-term storage, store at -20°C.

J. DNA Purification Protocol for 5 mL Whole Blood

Materials to be supplied by user

- Centrifuge capable of 2,000 x g
- Nuclease-free 15 mL centrifuge tubes
- Water baths preset at 37°C and 65°C
- Paper towels
- Isopropanol (100%)
- 70% ethanol

1. Add 5 mL whole blood (or bone marrow) to a nuclease-free 15 mL microcentrifuge tube containing 15 mL ERL Buffer. Mix by inverting the tube a few times. Incubate 5 minutes at room temperature. Invert the tube several times during incubation.

   NOTE: ERL Buffer is supplied as a 10x concentrate and must be diluted with ddH_2O according to bottle label or Page 4 above before use.

2. Centrifuge at 2,000 x g for 5 minutes at room temperature. Remove and discard as much supernatant as possible without disturbing the visible white pellet. Leave about 350 µL of residue liquid in the tube. If the blood sample has been frozen, repeat Steps 1-2 until the pellet is white.

   Note: If some red blood cells or cell debris are still visible along with the white blood cell pellet, resuspend the white blood cell pellet and mix with 2 volumes ERL Buffer. Incubate 2 min at room temperature. Then pellet the white blood cells by repeating Step 2.

3. Vortex the tube vigorously until the white blood cells are completely re-suspended.

4. Add 5 mL WTL Buffer to the tube containing the re-suspended cells. Pipet up and down to lyse the cells. The solution should become very viscous. If cell clumps are visible after mixing, incubate the solution at 37°C until the clumps cannot be seen.

5. **(Optional)** Add 25 µL RNase A solution to the cell lysate. Mix the sample by inverting the tube 20-25 times. Incubate the mixture at 37°C for approximately 10 minutes.

6. Cool the sample to room temperature.

7. Add 1.70 mL PCP Buffer to the cell lysate.

8. Vortex vigorously at high speed for 30 seconds to mix. Some protein clumps may be visible after vortexing.

9. Centrifuge at 2,000 x g or higher for 5 minutes at room temperature. The precipitated protein will form a tight, dark brown pellet. If the pellet is not tight or visible, repeat Step 8, incubate the tube in ice for 5 minutes, and then repeat Step 9.

10. Transfer the supernatant to a new nuclease-free 15 mL centrifuge tube containing 5 mL of 100% isopropanol. Do not transfer the protein pellet.

11. Gently mix the solution by inverting the tube 40-50 times.

12. Centrifuge at 2,000 x g for 3 minutes at room temperature. DNA will be visible as a small white pellet.

13. Pour out the supernatant and drain the tube briefly on an absorbent paper towel. Add 5 mL of 70% ethanol and invert the tube few times to wash the DNA pellet.

14. Centrifuge at 2,000 x g for 2 minutes at room temperature. Carefully pour out the ethanol. **Pellet may be very loose at this point, so pour slowly and be careful**

15. Invert the tube on an adsorbent paper towel and air dry the pellet for 10-15 minutes.

16. Add 500 µL of Buffer EB and vortex for 1 minute to mix.

17. Incubate sample at 65°C for 1 hour and overnight at room temperature to rehydrate DNA. Gently shake tube several times during incubation to disperse DNA.

18. Store DNA at 2-8°C. For long-term storage, store at -20°C.

K. DNA Purification Protocol for 6 mL Whole Blood

Materials to be supplied by user

- Centrifuge capable of 2,000 x g
- Nuclease-free 15 mL centrifuge tubes
- Water baths preset at 37°C and 65°C
- Paper towels
- Isopropanol (100%)
- 70% ethanol

1. Add 6 mL whole blood (or bone marrow) to a nuclease-free 50 mL microcentrifuge tube containing 18 mL ERL Buffer. Mix by inverting the tube a few times. Incubate 5 minutes at room temperature. Invert the tube several times during incubation.

   NOTE: ERL Buffer is supplied as a 10x concentrate and must be diluted with ddH_2O according to bottle label or instruction on Page 4 above before use.

2. Centrifuge at 2,000 x g for 5 minutes at room temperature. Remove and discard as much supernatant as possible without disturbing the visible white pellet. Leave about 400 µL of residue liquid in the tube. If the blood sample has been frozen, repeat Steps 1-2 until the pellet is white.

   Note: If some red blood cells or cell debris are still visible along with the white blood cell pellet, resuspend the white blood cell pellet and mix with 2 volumes ERL Buffer. Incubate 2 min at room temperature. Then pellet the white blood cells by repeating Step 2.

3. Vortex the tube vigorously until the white blood cells are completely re-suspended.

4. Add 6 mL WTL Buffer to the tube containing the re-suspended cells. Pipet up and down to lyse the cells. The solution should become very viscous. If cell clumps are visible after mixing, incubate the solution at 37°C until the clumps cannot be seen.

5. **(Optional)** Add 30 µL RNase A solution to the cell lysate. Mix the sample by inverting the tube 20-25 times. Incubate the mixture at 37°C for approximately 10 minutes.

6. Cool the sample to room temperature.

7. Add 2.0 mL PCP Buffer to the cell lysate.

8. Vortex vigorously at high speed for 30 seconds to mix. Some protein clumps may be visible after vortexing.

9. Centrifuge at 2,000 x g or higher for 5 minutes at room temperature. The precipitated protein will form a tight, dark brown pellet. If the pellet is not tight or visible, repeat Step 8, incubate the tube in ice for 5 minutes, and then repeat Step 9.

10. Transfer the supernatant to a new nuclease-free 15 mL centrifuge tube containing 6 mL of 100% isopropanol. Do not transfer the protein pellet.

11. Gently mix the solution by inverting the tube 40-50 times.

12. Centrifuge at 2,000 x g for 3 minutes at room temperature. DNA will be visible as a small white pellet.

13. Pour out the supernatant and drain the tube briefly on an absorbent paper towel. Add 6 mL of 70% ethanol and invert the tube few times to wash the DNA pellet.

14. Centrifuge at 2,000 x g for 2 minutes at room temperature. Carefully pour out the ethanol. **Pellet may be very loose at this point, so pour slowly**

15. Invert the tube on an adsorbent paper towel and air dry the pellet for 10-15 minutes.

16. Add 600 µL of Buffer EB and vortex for 1 minute to mix.

17. Incubate sample at 65°C for 1 hour and overnight at room temperature to rehydrate DNA. Gently shake tube several times during incubation to disperse DNA.

18. Store DNA at 2-8°C. For long-term storage, store at -20°C.

L. DNA Purification Protocol for 12 mL Whole Blood

Materials to be supplied by user

- Centrifuge capable of 2,000 x g
- Nuclease-free 50 mL centrifuge tubes
- Water baths preset at 37°C and 65°C
- Paper towels
- Isopropanol (100%)
- 70% ethanol

1. Add 12 mL whole blood (or bone marrow) to a nuclease-free 50 mL microcentrifuge tube containing 36 mL ERL Buffer. Mix by inverting the tube 5 times. Incubate 5 minutes at room temperature. Invert the tube once every minute during incubation.

   NOTE: ERL Buffer is supplied as a 10x concentrate and must be diluted with ddH_2O according to bottle label before use.

2. Centrifuge at 2,000 x g for 5 minutes at room temperature. Remove and discard as much supernatant as possible without disturbing the visible white pellet. Leave about 350 µL of residue liquid in the tube. If the blood sample has been frozen, repeat Steps 1-2 until the pellet is white.

   Note: If some red blood cells or cell debris are still visible along with the white blood cell pellet, resuspend the white blood cell pellet and mix with 2 volumes ERL Buffer. Incubate 2 min at room temperature. Then pellet the white blood cells by repeating Step 2.

3. Vortex the tube vigorously until the white blood cells are completely resuspended.

4. Add 12 mL WTL Buffer to the tube containing the resuspended cells. Vortex for 2 minutes to lyse the cells. The solution should become very viscous. If cell clumps are visible after mixing, incubate the solution at 37°C until the clumps cannot be seen.

5. **(Optional)** Add 50 µL RNase A solution to the cell lysate. Mix the sample by inverting the tube 20-25 times. Incubate the mixture at 37°C for approximately 10 minutes.

6. Cool the sample to room temperature.

7. Add 4.0 mL PCP Buffer to the cell lysate.

8. Vortex vigorously at high speed for 30 seconds to mix. Some protein clumps may be visible after vortexing.

9. Centrifuge at 2,000 x g or higher for 10 minutes at room temperature. The precipitated protein will form a tight, dark brown pellet. If the pellet is not tight or visible, repeat Step 8, incubate the tube in ice for 5 minutes, and then repeat Step 9.

10. Transfer the supernatant to a new nuclease-free 50 mL centrifuge tube containing 12 mL of 100% isopropanol. Do not transfer the protein pellet.

11. Gently mix the solution by inverting the tube 40-50 times.

12. Centrifuge at 2,000 x g for 5 minutes at room temperature. DNA will be visible as a small white pellet.

13. Pour out the supernatant and drain the tube briefly on an absorbent paper towel. Add 12 mL of 70% ethanol and invert the tube 5 times to wash the DNA pellet.

14. Centrifuge at 2,000 x g for 5 minutes at room temperature. Carefully pour out the ethanol. **Pellet may be very loose at this point, so pour slowly and be careful not to pour out the pellet.**

    Note: If the resulting pellets are loose, centrifuge at higher speed or with prolonged time.

15. Invert the tube on an adsorbent paper towel and air dry the pellet for 10-15 minutes.

16. Add 1mL of Buffer EB and vortex for 1 minute to mix.

17. Incubate sample at 65°C for 1 hour and room temperature for overnight to rehydrate DNA. Gently shake tube several times during incubation to disperse DNA.

18. Store DNA at 2-8°C. For long-term storage, store at -20°C.

M. DNA Purification Protocol for Buffy Coat (Prepared from 1-1.5 mL whole blood)

Materials to be supplied by user

- Microcentrifuge capable of 14,000 x g
- Nuclease-free 2.0 mL microcentrifuge tubes
- Water Bath preset at 37°C and 65°C
- Isopropanol
- 70% ethanol

The buffy coat fraction of whole blood is enriched with WBC, and usually gives at least 5-fold more DNA than the same volume of blood. To prepare buffy coat from fresh whole blood, simply centrifuge the sample at 3,000-4,000 x g for 10 min at room temperature. Three layers should be obtained, with plasma in the upper layer, leucocytes in the middle layer (buffy coat), and erythrocytes in bottom layer. Carefully aspirate the plasma, making sure not to disturb the layer of concentrated leukocytes. The buffy coat can be drawn off with a pipette and used directly in the E.Z.N.A.® Blood DNA Protocol, or frozen at -70°C for storage.

1. Add 75-120 µl buffy coat preparation (prepared from 1.5 mL whole blood) to a nuclease-free 2 mL microcentrifuge tube containing 400µL ERL Buffer. Mix by inverting the tube a few times. Incubate 5 minutes at room temperature. Invert the tube several times during incubation.

2. Centrifuge at 14,000 x g for 30 seconds at room temperature. Remove and discard as much supernatant as possible without disturbing the visible white pellet. Leave about 15µL of residue liquid in the tube. If the blood sample has been frozen, repeat Steps 1-2 until the pellet is white.

   Note: If some red blood cells or cell debris are still visible along with the white blood cell pellet, resuspend the white blood cell pellet and mix with 2 volumes ERL Buffer. Incubate 2 min at room temperature. Then pellet the white blood cells by repeating Step 2.

3. Vortex the tube vigorously until the white blood cells are completely resuspended.

4. Add 1.3 mL WTL Buffer to the tube containing the resuspended cells. Pipet up and down to lyse the cells. The solution should become very viscous. If cell clumps are visible after mixing, incubate the solution at 37°C until the clumps cannot be seen.

5. **(Optional)** Add 5 µL RNase A solution to the cell lysate. Mix the sample by inverting the tube 20-25 times. Incubate the mixture at 37°C for 5-10 minutes.

6. Cool the sample to room temperature.

7. Add 433µL PCP Buffer to the cell lysate.

8. Vortex vigorously at high speed for 30 seconds to mix. Some protein clumps may be visible after vortexing. Incubate in ice for 5 minutes.

9. Centrifuge at max speed for 3 minutes at room temperature. The precipitated protein will form a tight, dark brown pellet. If the pellet is not tight or visible, incubate the tube in ice for 5 minutes and repeat Step 9.

10. Transfer the supernatant to a new nuclease-free 2.0 mL centrifuge tube containing 1.3 mL of 100% isopropanol.

11. Gently mix the solution by inverting the tube 30-40 times.

12. Centrifuge at 14,000 x g for 1 minute at room temperature. DNA will be visible as a small white pellet.

13. Pour out the supernatant and drain the tube briefly on a clean absorbent paper towel. Add 1.3 mL of 70% ethanol and invert the tube a few times to wash the DNA pellet.

14. Centrifuge at 14,000 x g for 2 minutes at room temperature. Carefully pour off the ethanol. **Pellet may be very loose at this point, so pour slowly and watch the pellet.**

15. Invert the tube on a clean adsorbent paper towel and air dry the pellet for 10-15 minutes.

16. Add 500 µL of DNA rehydration solution (Buffer EB) and vortex for 1 minute to mix.

17. Incubate sample at 65°C for 1 hour and room temperature for overnight to rehydrate DNA. Gently shake tube several times during incubation to disperse DNA.

18. Store DNA at 2-8°C. For long-term storage, store at -20°C.

Reagent Cells	Number of Cells		
	0.5-1.0 x 10^6	3-5 x 10^6	3-5 x 10^7
WTL	150µL	600µL	6.0 mL
Rnase A	1µL	3 µL	30 µL
PCP	50µL	200 µL	2.0 mL
Isopropanol	150µL	600 µL	6.0 mL
70 % EtOH	150µL	600 µL	6.0 mL
Buffer EB	25µL	100µL	500µL

N. DNA Purification Protocol for Cultured Cells 0.5-1 x 10^6
Materials to be supplied by user

- Microcentrifuge capable of 14,000 x g
- Nuclease-free 2.0 mL microcentrifuge tubes
- Water Bath preset at 37°C and 65°C
- Isopropanol
- 70% ethanol

1. This protocol is designed for isolating genomic DNA from 0.5-1 million cultured cells.
2. Harvest the cells and transfer them with salt balanced buffer (such as PBS) to a 2.0 mL microcentrifuge tube. For adherent cells, trypsinize the cells before harvesting.
3. Centrifuge at 14,000 x g for 10 seconds to pellet the cells. Remove the cells and leave behind about 25 µL residue liquid.
4. Vortex the cells to resuspend the cells in the residue liquid. Make no cell clumps visible at this point.
5. Add 150µL of WTL Buffer to the resuspended cells and mix by pipetting. The solution should become very viscous. If cell clumps are visible after mixing, incubate the solution at 37°C until the clumps are cannot be seen.
6. **(Optional)** Add 1 µL RNase A solution to the cell lysate. Mix the sample by inverting the tube 20-25 times. Incubate the mixture at 37°C for 5-10 minutes.
7. Cool the sample to room temperature.
8. Add 50µL PCP Buffer to the cell lysate.
9. Vortex vigorously at high speed for 30 seconds to mix. Some protein clumps may be visible after vortexing. Incubate in ice for 5 minutes.
10. Centrifuge at max speed for 3 minutes at room temperature. The precipitated protein will form a tight pellet. If the pellet is not tight or visible, incubate the tube in ice for 5 minutes and repeat Step 9.
11. Transfer the supernatant to a new nuclease-free 2.0 mL centrifuge tube containing 150µL of 100% isopropanol.
12. Gently mix the solution by inverting the tube 30-40 times.
13. Centrifuge at 14,000 x g for 1 minute at room temperature. DNA will be visible as a small white pellet.
14. Pour out the supernatant and drain the tube briefly on a clean absorbent paper towel. Add 150µL of 70% ethanol and invert the tube a few times to wash the DNA pellet.
15. Centrifuge at 14,000 x g for 2 minutes at room temperature. Carefully pour off the ethanol. **Pellet may be very loose at this point, so pour slowly and watch the pellet.**
16. Invert the tube on a clean adsorbent paper towel and air dry the pellet for 10-15 minutes.
17. Add 25 µL of DNA rehydration solution (Buffer EB) and vortex for 1 minutes to mix.
18. Incubate sample at 65°C for 1 hour and room temperature for overnight to rehydrate DNA. Gently shake tube several times during incubation to disperse DNA.
19. Store DNA at 2-8°C. For long-term storage, store at -20°C.

P. DNA Purification Protocol for Cultured Cells 3-5 x 10^6
Materials to be supplied by user

- Microcentrifuge capable of 14,000 x g
- Nuclease-free 2.0 mL microcentrifuge tubes
- Water Bath preset at 37°C and 65°C
- Isopropanol
- 70% ethanol

1. This protocol is designed for isolating genomic DNA from **3-5 x 10^6** cultured cells.

2. Harvest the cells and transfer them with salt balanced buffer (such as PBS) to a 2.0 mL microcentrifuge tube. For adherent cells, trypsinize the cells before harvesting.

3. Centrifuge at 14,000 x g for 10 seconds to pellet the cells. Remove the cells and leave behind about 25 µL residue liquid.

4. Vortex the cells to resuspend the cells in the residue liquid. Make no cell clumps visible at this point.

5. Add 600 µL of WTL Buffer to the re-suspended cells and mix by pipetting. The solution should become very viscous. If cell clumps are visible after mixing, incubate the solution at 37°C until the clumps are cannot be seen.

6. **(Optional)** Add 3 µL RNase A solution to the cell lysate. Mix the sample by inverting the tube 20-25 times. Incubate the mixture at 37°C for 5-10 minutes.

7. Cool the sample to room temperature.

8. Add 200µL PCP Buffer to the cell lysate.

9. Vortex vigorously at high speed for 30 seconds to mix. Some protein clumps may be visible after vortexing. Incubate in ice for 5 minutes.

10. Centrifuge at max speed for 3 minutes at room temperature. The precipitated protein will form a tight pellet. If the pellet is not tight or visible, incubate the tube in ice for 5 minutes and repeat Step 9.

11. Transfer the supernatant to a new nuclease-free 2.0 mL centrifuge tube containing 600 µL of 100% isopropanol.

12. Gently mix the solution by inverting the tube 30-40 times.

13. Centrifuge at 14,000 x g for 1 minute at room temperature. DNA will be visible as a small white pellet.

14. Pour out the supernatant and drain the tube briefly on a clean absorbent paper towel. Add 600 µL of 70% ethanol and invert the tube a few times to wash the DNA pellet.

15. Centrifuge at 14,000 x g for 2 minutes at room temperature. Carefully pour off the ethanol. **Pellet may be very loose at this point, so pour slowly and watch the pellet.**

16. Invert the tube on a clean adsorbent paper towel and air dry the pellet for 10-15 minutes.

17. Add 100 µL of DNA rehydration solution (Buffer EB) and vortex for 1 minutes to mix.

18. Incubate sample at 65°C for 1 hour and room temperature for overnight to rehydrate DNA. Gently shake tube several times during incubation to disperse DNA.

19. Store DNA at 2-8°C. For long-term storage, store at -20°C

Q. DNA Purification Protocol for Cultured Cells 3-5 10^7

Materials to be supplied by user

- Centrifuge capable of 2,000 x g
- Nuclease-free 15 mL microcentrifuge tubes
- Water Bath preset at 37°C and 65°C
- Isopropanol
- 70% ethanol

1. This protocol is designed for isolating genomic DNA from 3-5 10^7 cultured cells.
2. Harvest the cells and transfer them with salt balanced buffer (such as PBS) to a15 mL microcentrifuge tube. For adherent cells, trypsinize the cells before harvesting.
3. Centrifuge at 500 x g for 3 minutes to pellet the cells. Remove the cells and leave behind about 2500 µL residue liquid.
4. Vortex the cells to resuspend the cells in the residue liquid. Make no cell clumps visible at this point.
5. Add 6000 µL of WTL Buffer to the re-suspended cells and mix by pipetting. The solution should become very viscous. If cell clumps are visible after mixing, incubate the solution at 37°C until the clumps are cannot be seen.
6. **(Optional)** Add 30 µL RNase A solution to the cell lysate. Mix the sample by inverting the tube 20-25 times. Incubate the mixture at 37°C for 5-10 minutes.
7. Cool the sample to room temperature.
8. Add 2000µL PCP Buffer to the cell lysate.
9. Vortex vigorously at high speed for 30 seconds to mix. Some protein clumps may be visible after vortexing. Incubate in ice for 5 minutes.
10. Centrifuge at 2000 x g for 10 minutes at room temperature. The precipitated protein will form a tight pellet. If the pellet is not tight or visible, incubate the tube in ice for 5 minutes and repeat Step 9.
11. Transfer the supernatant to a new nuclease-free 2.0 mL centrifuge tube containing 6000 µL of 100% isopropanol.
12. Gently mix the solution by inverting the tube 30-40 times.
13. Centrifuge at 2000 x g for 10 minutes at room temperature. DNA will be visible as a small white pellet.
14. Pour out the supernatant and drain the tube briefly on a clean absorbent paper towel. Add 6000 µL of 70% ethanol and invert the tube a few times to wash the DNA pellet.
15. Centrifuge at 2000 x g for 10 minutes at room temperature. Carefully pour off the ethanol. **Pellet may be very loose at this point, so pour slowly and watch the pellet.**
16. Invert the tube on a clean adsorbent paper towel and air dry the pellet for 10-15 minutes.
17. Add 1000 µL of DNA rehydration solution (Buffer EB) and vortex for 2 minutes to mix.
18. Incubate sample at 65°C for 1 hour and room temperature for overnight to rehydrate DNA. Gently shake tube several times during incubation to disperse DNA.
19. Store DNA at 2-8°C. For long-term storage, store at -20° C

R: DNA Purification from 50µL Clotted Blood

1 Transfer the 50µL blood include any liquid residual into a 1.5 mL centrifuge tube.

2 Add 550µL WTL Buffer and pipet up an down few times to mix.

3 Add 3 µL Proeinase K solution (25mg/mL) and mix by inverting 20 times.

4 Incubate at 55°C for 1 hour to overnight until clots has dissolved.

5 Place the tube on ice for 1 minute.

6 Add 3 µL RNase A to the cell lysate and invert 10 time to mix throughly. Incubate the tube at 37°C for 5 minutes.

7 Place the tube on ice for 1 minute.

8 Add 200 µL PCP buffer to the cell lysate.

9 Vortex vigorously at high speed for 30 seconds to mix. Some protein clumps may be visible after vortexing. Incubate in ice for 5 minutes.

10 Centrifuge at max speed for 3 minutes at room temperature. The precipitated protein will form a tight, dark brown pellet. If the pellet is not tight or visible, incubate the tube in ice for 5 minutes and repeat this step.

11 Transfer the supernatant to a new nuclease-free 2.0 mL centrifuge tube containing 600µL of 100% isopropanol. If the DNA yield is expected to be lower than 2µg, add 2µL of glycogen (20mg/mL) per sample.

12 Gently mix the solution by inverting the tube 30-40 times. Centrifuge at 14,000 x g for 1 minute at room temperature. DNA will be visible as a small white pellet.

13 Pour of the supernatant and drain the tube briefly on a clean absorbent paper towel. Add 600µL of 70% ethanol and invert the tube a few times to wash the DNA pellet.

14 Centrifuge at 14,000 x g for 2 minutes at room temperature. Carefully pour off the ethanol. **Pellet may be very loose at this point, so pour slowly and watch the pellet.**

15 Invert the tube on a clean adsorbent paper towel and air dry the pellet for 10-15 minutes.

16 Add 20 µL of DNA rehydration solution (Buffer EB) and vortex for 1 minute to mix.

17 Incubate sample at 65°C for 1 hour and room temperature for overnight to rehydrate DNA. Gently shake tube several times during incubation to disperse DNA.

18 Store DNA at 2-8°C. For long-term storage, store at -20°C.

S: DNA Purification from 1mL Clotted Blood

1 Transfer 1 mL clotted blood include any liquid residual into a 50 mL centrifuge tube.

2 Add 11 mL WTL Buffer and pipet up an down few times to mix.

3 Add 50 µL Proteinase K solution (25mg/mL) and mix by inverting 20 times.

4 Incubate at 55°C for 3 hour to overnight until clots has dissolved.

5 Place the tube on ice for 1-2 minute.

6 Add 50 µL RNase A to the cell lysate and invert 10 time to mix throughly. Incubate the tube at 37°C for 5 minutes.

7 Place the tube on ice for 1-2 minute.

8 Add 4 mL PCP buffer to the cell lysate.

9 Vortex vigorously at high speed for 30 seconds to mix. Some protein clumps may be visible after vortexing. Incubate in ice for 10 minutes.

10 Centrifuge at 2000 x g for 10 minutes at room temperature. The precipitated protein will form a tight, dark brown pellet. If the pellet is not tight or visible, incubate the tube in ice for 5 minutes and repeat this step.

11 Transfer the supernatant to a new 50 mL centrifuge tube containing12 mL of 100% isopropanol. Add 20µL of glycogen (20mg/mL) per sample.

12 Gently mix the solution by inverting the tube 30-40 times. Centrifuge at 2000 x g for 5 minute at room temperature. DNA will be visible as a small white pellet.

13 Pour of the supernatant and drain the tube briefly on a clean absorbent paper towel. Add 12mL of 70% ethanol and invert the tube a 5 times to wash the DNA pellet.

14 Centrifuge at 2000 x g for 2 minutes at room temperature. Carefully pour off the ethanol. **Pellet may be very loose at this point, so pour slowly and watch the pellet.**

15 Invert the tube on a clean adsorbent paper towel and air dry the pellet for 10-15 minutes.

16 Add 400 µL of DNA rehydration solution (Buffer EB) and vortex for 1 minute to mix.

17 Incubate sample at 65°C for 1 hour and room temperature for overnight to rehydrate DNA. Gently shake tube several times during incubation to disperse DNA.

18 Store DNA at 2-8°C. For long-term storage, store at -20°C.

Q : DNA Purification from large volume (>1mL) Clotted Blood

1. Transfer the clotted blood include any liquid residual into a 50 mL centrifuge tube.

2. Homogenize the sample with a rotor-stator homogenizer until the sample is uniformly homogenous

3. Add 3 volume of 1 x ERL buffer and mix by invert the tube 5-7 times.

4. Incubate at RT for 5 minutes.

5. Centrifuge at 2000 x g for 5 minutes

6. Discard the supernatant and leave the tube inverted on a clean absorbent paper for 2 minutes. Make sure that the pellet remain in the tube.

7. Add 5mL WTL Buffer and 50 µL Proteinase K solution (25mg/mL), close the cap and vortex immediately until the pellet is completely homogenized.

   Note: When processing multiple samples, vortex each tube immediately after addition of WTL Buffer/ Proteinase K . Do not wait until buffer has been added to all samples before vortexing. Although the pellet can be easily homogenized with 3-4 pulses of high-speed vortexing, however, traces of pellet with a jelly-like consistency (often barely visible) may remain. If these traces are seen, vortex sample for another 30 seconds.

8. Incubate the tube at 65°C for 30 minutes in a water bath or heating block.

9. After the lysate cool to room temperature, add 1.7 mL PCP buffer. Mix by vortexing for 15 seconds.

10. Incubate at ice for 10 minutes.

11. Centrifuge at 2000-4000 x g for 10 minutes to pellet the protein.

12. Transfer the supernatant to a new 50 mL tube, add 4.75mL of isopropanol and mix gently by invert the tube 20-30 times. DNA precipitate will become visible as threads or a clump.

13. Centrifuge at 2000 -4000 x g for 10 minutes to pellet the DNA.

14. Discard the supernatant and briefly invert the tube onto a clean absorbent paper, make sure the DNA pellet remains inside the tube.

15. Add 5 mL 70% ethanol and vortex for 10 seconds.

16. Centrifuge at 2000-4000 x g for 5-10 minutes.

17. Discard the supernatant and invert the tube onto a clean absorbent paper for 5 minutes, make sure the DNA pellet remains inside the tube.

18. Air dry the DNA pellet until all liquid are evaporated. Do not over dry the pellet because it is very difficult to dissolve the over-dried DNA pellet.

19. Add 1 mL EB Buffer or TE Buffer, vortex 5 seconds at lower speed. Dissolve the DNA by incubating 1 hour at 65C and overnight at room temperature.

Determination of Yield and Quality

The total DNA yield can be determined by a spectrophotometer using deionized water, Tris-HCl buffer, or Elution Buffer as blank. Dilute the DNA in TE buffer and calculate concentration as:

[DNA] = (Absorbance$_{260}$) x (0.05 µg/µL) x (Dilution factor)

The quality of DNA can be assessed by measuring absorbance at both 260 nm and at 280 nm. A ratio of (A_{260}/A_{280}) of 1.7-1.9 corresponds to 85%-95% purity.

Expected yields range from 4 µg to 12 µg DNA per 250 µL whole blood, depending on source of sample, its age, and the method of storage. Yields are generally 5-fold higher with Buffy Coat samples.

Troubleshooting Guide

Problem	Possible Cause	Suggestions
Low DNA yield	Blood Sample contains too few white blood cells	Draw new blood samples
	Blood sample is too old.	Try to use fresh blood if possible.
	Incompletely re-suspended white blood cell pellet before adding WTL buffer.	Vortex vigorously to completely resuspend white blood cell.
	DNA pellet was lost during isopropanol precipitation	Be very careful not to lose the DNA when removing isopropanol or ethanol during precipitation and wash steps.
Low A_{260}/A_{280} ratio	The sample was not cooled to room temperature before adding PCP buffer	Cool the sample to room temperature or chill on ice for at least 5 minutes before adding PCP buffer.
	Poor cell lysis due to incomplete mixing with Buffer WTL	Repeat the procedure, this time making sere to vortex the sample with Buffer WTL immediately and completely.
	Hemoglobin remains	Repeat the procedure, this time making sure enough volume of ERL is used and white blood cell pellet is white in color .
	PCP Buffer was not mixed with WTL buffer throughly.	Make sure that PCP buffer and cell lysate is mixed throughly.
No DNA	DNA pellet was lost during isopropanol precipitation	Be very careful not to lose the DNA when removing isopropanol or ethanol during precipitation and wash steps.
DNA Pellet is difficult to dissolve	DNA pellet was over dried	Rehydrate the DNA by incubating the DNA pellet with EB Buffer at 65°C for 1 hour and then leave the sample at room temperature or 4°C for overnight.
	DNA pellet was not mixed well during rehydration step.	Shake a few times during the rehydration step.